

What Is Claimed Is:

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1. An expression cassette comprising,
a polynucleotide encoding *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products, wherein (a) the arrangement of coding sequences for the gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE*- 3'; (b) transcription of the polynucleotide results in a polycistronic RNA encoding all the gene products; and (c) each of the *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products is expressed as an individual polypeptide.
 2. The expression cassette of claim 1, wherein a multiple-insertion site is located adjacent the 5' end of the *luxA* coding sequences.
 3. The expression cassette of claim 1, further comprising at least one Gram-positive ribosome binding site sequence (SEQ ID NO:1) upstream of each of the polynucleotide sequences encoding each of the *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products.
 4. The expression cassette of claim 1, wherein the coding sequences of the gene products are derived from *Photorhabdus luminescens*.
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5. The expression cassette of claim 5, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sa1-Sa6.
 6. The expression cassette of claim 5, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sa2 and Sa4.
 7. The expression cassette of claim 1, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

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8. The expression cassette of claim 7, wherein transcription of the polynucleotide is mediated by a promoter contained in Expression Enhancing Sequence Sp16.

9. An expression cassette comprising,
a polynucleotide encoding *luxA*, and *luxB* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding both gene products, and (b) polynucleotide sequences comprising Gram-positive ribosome-binding site sequences are located adjacent the 5' end of the *luxA* coding sequences and adjacent the 5' end of the *luxB* coding sequences.

10. The expression cassette of claim 9, further comprising an insertion site 5' to at least one of either the *luxA* or *luxB* coding sequences.

11. The expression cassette of claim 10, wherein the insertion site further comprises a multiple-insertion site.

12. The expression cassette of claim 11, wherein the multiple-insertion site is located 5' to the *luxA* coding sequences.

13. The expression cassette of claim 9, wherein said polynucleotide further encodes *luxC*, *luxD* and *luxE* gene products.

14. The expression cassette of claim 12, wherein the arrangement of coding sequences for the *lux* gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE*- 3'.

15. The expression cassette of claim 12, wherein Gram-positive bacterial Shine-Dalgarno sequences are 5' to all of said *lux* coding sequences.

16. The expression cassette of claim 12, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sa1-Sa6.

17. The expression cassette of claim 16, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sa2 and Sa4.

18. The expression cassette of claim 12, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

19. The expression cassette of claim 18, wherein transcription of the polynucleotide is mediated by a promoter contained in Expression Enhancing Sequence Sp16.

20. The expression cassette of claim 9, wherein the coding sequences for *luxA* and *luxB* are obtained from *Photobacterium luminescens*.

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21. An expression cassette comprising,
a polynucleotide encoding *luxA*, *luxB*, and *luc* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding all three gene products, and (b) polynucleotide sequences comprising Gram-positive bacterial Shine-Dalgarno sequences are located adjacent the 5' end of the *luxA* coding sequences, adjacent the 5' end of the *luxB* coding sequences, and adjacent the 5' end of the *luc* coding sequences,

22. The expression cassette of claim 21, wherein said polynucleotide further encodes *luxC*, *luxD* and *luxE* gene products.

23. The expression cassette of claim 22, wherein Gram-positive bacterial Shine-Dalgarno sequences are located 5' to all of the *lux* coding sequences.

24. The expression cassette of claim 21, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sa1-Sa6.

25. The expression cassette of claim 24, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sa2 and Sa4.

26. The expression cassette of claim 21, wherein transcription of the polynucleotide is mediated by a promoter contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

27. The expression cassette of claim 26, wherein transcription of the polynucleotide is mediated by a promoter contained in Expression Enhancing Sequence Sp16.

28. The expression cassette of claim 21, wherein a multiple-insertion site is located adjacent the 5' end of the *luxA* coding sequences.

29. The expression cassette of claim 21, wherein the coding sequences for *luxA* and *luxB* are obtained from *Photobacterium luminescens*.

30. An expression cassette comprising,
a polynucleotide encoding an in-frame fusion of *luxA* and *luxB* gene products, wherein
(a) polynucleotide sequences comprising Gram-positive Shine-Dalgarno sequences are located adjacent the 5' end of the *luxA* coding sequences, and (b) an insertion site is located between the *luxA* and *luxB* coding sequences.

31. The expression cassette of claim 30, wherein the insertion site further comprises a multiple-insertion site.

32. The expression cassette of claim 30, wherein said polynucleotide further encodes *luxC*, *luxD* and *luxE* gene products, wherein the arrangement of coding sequences for the gene products is in the following relative order 5' - *luxA/luxB-luxC-luxD-luxE*- 3'

33. The expression cassette of claim 32, wherein Gram-positive bacterial Shine-Dalgarno sequences are 5' to the *luxA/luxB* fusion coding sequences and all of the *luxC*, *luxD*, and *luxE* coding sequences.

34. The expression cassette of claim 1, wherein the expression cassette is contained within a bacterial transposon.

35. The expression cassette of claim 1, wherein the expression cassette is contained within a bacterial mini-transposon.

36. The expression cassette of claim 34, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced.

37. A method of selecting a light-producing expression cassette for use in a selected cell type, said method comprising
preparing fragments of genomic DNA isolated from the selected cell type,
inserting the fragments into the insertion site of an expression cassette of any of claims 30, where the expression cassette is capable of expressing the gene products in the selected cell type,
introducing the expression cassettes carrying the fragments into cells of the selected cell type, and
screening for cells producing light, where said light production is mediated by the expression cassette.

38. The method of claim 37, where said fragments are produced by enzymatic digestion of genomic DNA.

39. The method of claim 38, where said fragments are produced by partial digestion using a selected restriction endonuclease.

40. The method of claim 37, where said fragments are produced by mechanical fragmentation of the genomic DNA.

41. The method of claim 37, wherein transcription of the *lux* genes is mediated by a promoter that is obtained from the selected cell type.

42. The method of claim 37, wherein the selected cell type is selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Actinomyces*, *Lactobacillus*, *Corynebacterium*, *Mycobacterium*, *Clostridium*, *Propionibacterium*, *Enterococcus*, and *Bacillus*.

43. The method of claim 37, where said screening is carried out at a temperature greater than 37°C.

44. A luciferase expression cassette comprising:
a) a polynucleotide encoding *luc*; and
b) polynucleotide sequences comprising expression enhancing sequences obtained from Gram-positive bacteria 5' to said *luc*-encoding polynucleotide.

45. The expression cassette of claim 44 wherein the expression enhancing sequences are Gram-positive Shine-Dalgarno sequences.

46. The expression cassette of either of claim 44 wherein the expression enhancing sequences are Gram-positive promoter sequences.

47. The expression cassette of claim 44, wherein the small DNA fragment is between *luc* and the promoter and wherein the small DNA fragment is selected from the group

consisting of a nucleotide encoding an open-reading frame of the iron transport protein of *Staphylococcus* a polynucleotide encoding an open-reading frame of the alanine-racine operon and a polynucleotide encoding an open-reading frame a protein having homology to a *Bacillus* protein.

48. The plasmids designated pCMOR G+1 Sa1-6 and pCMOR G+2 Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

49. A shuttle vector comprising:

- a) an expression cassette according to claim 1;
- b) a polynucleotide encoding a selectable marker;
- c) a Gram-positive origin of replication; and
- d) a Gram-negative origin of replication.

50. A method of screening for expression enhancing sequences that are useful in obtaining expression of luciferase in Gram-positive bacteria, comprising:

a) introducing DNA fragments from a Gram-positive bacterial genome into an expression cassette comprising (i) polynucleotides encoding *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products, where the polynucleotides are in the following relative order 5' - *luxABCDE*; (ii) polynucleotide sequences comprising expression enhancing sequences obtained from Gram-positive bacteria 5' to at least one of said *lux*-encoding polynucleotides and (iii) an insertion site 5' to at least one of said *lux*-encoding polynucleotides;

b) transforming the expression cassette of step (a) into a Gram-positive bacteria host cells; and

c) determining the level of luciferase activity in the host cell, thereby identifying Gram-positive expression enhancing DNA sequences that are useful in obtaining expression of luciferase in Gram-positive bacteria.

51. A method of screening for expression enhancing sequences that are useful in obtaining expression of luciferase in Gram-positive bacteria, comprising:

a) introducing DNA fragments from a Gram-positive bacterial genome into an expression cassette comprising (i) polynucleotides encoding *luxA*, *luxB* gene products (ii) polynucleotide sequences comprising expression enhancing sequences obtained from Gram-positive bacteria 5' to at least one of said *lux*-encoding polynucleotides and (iii) an insertion site 5' to at least one of said *lux*-encoding polynucleotides;

b) transforming the expression cassette of step (a) into a Gram-positive bacteria host cells; and

c) determining the level of luciferase activity in the host cell, thereby identifying Gram-positive expression enhancing DNA sequences that are useful in obtaining expression of luciferase in Gram-positive bacteria.

52. A method of screening for expression enhancing sequences that are useful in obtaining expression of luciferase in Gram-positive bacteria, comprising:

a) introducing DNA fragments from a Gram-positive bacterial genome into an expression cassette comprising (i) a polynucleotide encoding *luc*; (ii) polynucleotide sequences comprising expression enhancing sequences obtained from Gram-positive bacteria 5' to said *luc*-encoding polynucleotide and (iii) an insertion site 5' to at least one of said *luc*-encoding polynucleotide;

b) transforming the expression cassette of step (a) into a Gram-positive bacteria host cells; and

c) determining the level of luciferase activity in the host cell, thereby identifying Gram-positive expression enhancing DNA sequences that are useful in obtaining expression of luciferase in Gram-positive bacteria

53. A method of making a luciferase expression cassette, comprising the steps of:

(a) preparing polynucleotides encoding in a 5'-3' direction *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products; and Gram-positive Shine-Dalgarno nucleotide sequences operably linked to one or more of said *lux*-encoding polynucleotides; and

(b) inserting small sequences of nucleic acids between one or more of the polynucleotides encoding a *lux* gene product.

54. A method of making a luciferase expression cassette, comprising the steps of:
(a) preparing polynucleotides encoding *luxA* and *luxB* gene products; and Gram-positive Shine-Dalgarno nucleotide sequences operably linked to one or more of said *lux*-encoding polynucleotides; and
(b) inserting small sequences of nucleic acids between one or more of the polynucleotides encoding a *lux* gene product.

55. A method of making a luciferase expression cassette, comprising the steps of:
(a) preparing polynucleotides encoding *luc* gene product; and Gram-positive Shine-Dalgarno nucleotide sequences operably linked to said *luc*-encoding polynucleotide; and
(b) inserting small sequences of nucleic acids 5' to said *luc*-encoding polynucleotide.

56. A method of modifying a Gram-positive organism to produce light, comprising transforming the Gram-positive organism with an expression cassette according to claim 1.

57. The method of claim 56 further comprising providing, if necessary, the substrate required for luciferase activity.

58. A method of screening an analyte for its ability to affect expression of a reporter marker, comprising:
(a) transforming Gram-positive bacteria with a luciferase expression cassette according to claim 4;
(b) providing the analyte to the bacteria;
(c) providing, if necessary, the substrate required for luciferase light production; and
(d) monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

59. The method of claim 58, wherein the substrate is aldehyde and is provided as a vapor.

sub A8 60. A method of screening an analyte for its ability to affect expression of a reporter marker in a whole animal, comprising:

- (a) transforming Gram-positive bacteria with a luciferase expression cassette according to claim 1;
- (b) introducing the bacteria into a whole animal;
- (c) providing the analyte to the animal;
- (d) providing, if necessary, the substrate required for luciferase light production; and
- (e) monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

61. The method of claim 60, wherein the substrate is aldehyde and is provided by injection.

sub A9 62. A Gram-positive bacteria capable of producing light, wherein (a) the bacteria comprise *luxA* and *luxB* coding sequences, and (b) about 1×10^6 bacterial cells can produce at least about 1×10^4 Relative Light Units at about 37°C.

63. A transgenic ~~non-human~~ animal comprising an expression cassette according to claim 1.

sub A10 64. A bacteria comprising an expression cassette according to claim 1.

65. The bacteria according to claim 64, wherein the bacteria is gram-positive.

66. A bacteria comprising a ~~plasmid~~ according to claim 48.

67. The bacteria according to claim 66, wherein the bacteria is gram-positive.

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